REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-98-

GSICI

ining the data
cing this
ement and

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 06/05/98	3. REPORT ... ..D DATES COVERED Final Technical 4/1/97 - 3/31/98

4. TITLE AND SUBTITLE

DURIP97 Instrumentation for Characterization of Two-Photon Absorbing Organic Materials

5. FUNDING NUMBERS

AFOSR Grant F49620-97-1-0200

3484/US

61103D

6. AUTHOR(S)

Dr. Seth Marder and Dr. Joseph Perry

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Beckman Institute m/c 139-74
California Institute of Technology
Pasadena, CA 91125

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

AFOSR/NL
110 Duncan Avenue, Room B115
Bolling AFB DC 20332-8050

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

19980630 049

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

DURIP funds were used to purchase a Spex Model FL112 High Performance Research Grade Fluorimeter and a Nd:YAG-pumped nanosecond Optical Parametric Oscillator Laser System with tunability from 440 nm - 1800 nm. This equipment was used to measure the two-photon absorption spectra for a variety of molecules from different classes and identify promising candidate molecules to meet DoD requirements for a variety of applications. A strategy for the design of molecules with large two-photon absorption cross sections has been developed. The strategy is based on the concept that symmetric charge transfer, from the ends of a conjugated system to the middle, or vice versa, upon excitation is correlated to enhanced two-photon absorption cross sections. It was shown that newly synthesized bis-styrylbenzene derivatives with donor-acceptor-donor, and acceptor-donor-acceptor structural motifs exhibit exceptionally large two-photon absorption cross sections, up to roughly 400 times that of *trans*-stilbene.

14. SUBJECT TERMS

Laser spectroscopy, organic dyes, multi-photon absorption, two-photon absorption, fluorescence, nonlinear excitation.

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

DTIC QUALITY INSPECTED 1

INSTRUMENTATION FOR CHARACTERIZATION OF TWO-PHOTON ABSORBING ORGANIC MATERIALS.

FINAL TECHNICAL REPORT FOR:

THE AIR FORCE OFFICE OF SCIENTIFIC RESEARCH:
DEFENSE UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
GRANT #F49620-97-1-0200

SETH R. MARDER AND JOSEPH W. PERRY

The Beckman Institute
California Institute of Technology
Pasadena, California 91125

Introduction

In the presence of intense laser pulses, molecules can simultaneously absorb two or more photons, making a transition to an electronically excited state which is higher in energy than the ground state by the sum of energies of the absorbed photons. The transition probability for absorption of two identical photons is proportional to I^2, where I is the intensity of the laser pulse. Molecules with a large two-photon absorption cross-section, δ, are in great demand for variety of applications including, two-photon excited fluorescence microscopy (*1- 4*), optical limiting (*5 - 7*), three-dimensional optical data storage (*8, 9*), and two-photon induced biological caging studies (*10*). These applications utilize two key features of two-photon absorption, namely, the ability to create excited states using photons of half the nominal excitation energy, which can provide improved penetration in absorbing or scattering media, and the I^2 dependence of the process which allows for excitation of chromophores with a high degree of spatial selectivity in three dimensions using a tightly focused laser beam. Unfortunately, most known organic molecules have relatively small δ, and criteria for the rational design of molecules with large δ have not been well developed (*11, 12*). As a result, the full utility of two-photon absorbing materials has not been realized. Here we report on design strategies and structure/property studies for two-photon absorption, that have lead to fluorescent molecules with unprecedented δ values, which should have a major impact on two-photon fluorescence imaging and other applications involving two-photon excitation.

Summary of research projects on which the equipments was used.

Initial optical studies revealed that 4,4'-bis(di-*n*-butyl)amino-*E*- stilbene, **2** (Figure 1), in toluene solution, exhibited a strong blue fluorescence when exposed to 5-ns laser pulses at 605 nm. The fluorescence intensity was found to depend on I^2, indicative of two-photon excited fluorescence. Compound **2** has a linear absorption peak at 385 nm, an

emission maximum at 410 nm, and a fluorescence quantum yield, Φ_f, of 0.90. The two-photon excited fluorescence spectrum for **2** was essentially identical to that excited by one-photon absorption into S_1, suggesting that there was rapid relaxation of state reached by two-photon absorption (taken to be S_2) to the S_1 state, and subsequent fluorescence from that state. Measurement of the two-photon excitation cross section for **2** gave a maximum δ of 210 x 10^{-50} cm^{4}sec/photon at an excitation wavelength of 605 nm, which is almost 20 times larger than that of *trans*-stilbene, **1**, (*13*) and is amongst the largest values of δ reported for organic compounds. We conjectured that the large increase in the two-photon absorption for **2** relative to **1** was related to a symmetrical charge transfer from the amino nitrogens to the conjugated bridge of the molecule.

In order to gain insight into the origin of the large δ value for **2** relative to **1**, we performed quantum-chemical calculations on **1** and 4,4'-bis(dimethyl)amino-*E*- stilbene. Using AM1 (*14*) optimized geometries, the energies (E) and transition dipole moments (M) for the singlet excited states of both compounds were calculated by combining the Intermediate Neglect of Differential Overlap (INDO) (*15*) Hamiltonian with the Multi-Reference Double Configuration Interaction (MRD-CI) (*16*) scheme. The frequency dependence of δ, $\delta(\omega)$, is related to the imaginary part of the second hyperpolarizability, Im $\gamma(-\omega;\omega,-\omega,\omega)$ by:

$$\delta(\omega) \quad = \quad L^4 \text{ Im } \gamma(-\omega;\omega,-\omega,\omega) \tag{1}$$

where $\hbar$ is Planck's constant/2π, n is the index of refraction the medium (vacuum assumed for the calculations), L is a local field factor (equal to 1 for vacuum), and c is the speed of light (*17*). We calculated Im $\gamma(-\omega;\omega,-\omega,\omega)$ using the Sum-Over-States (SOS) (*18*) expression (the damping factor, Γ, has been set to 0.1 eV in all cases in this study).
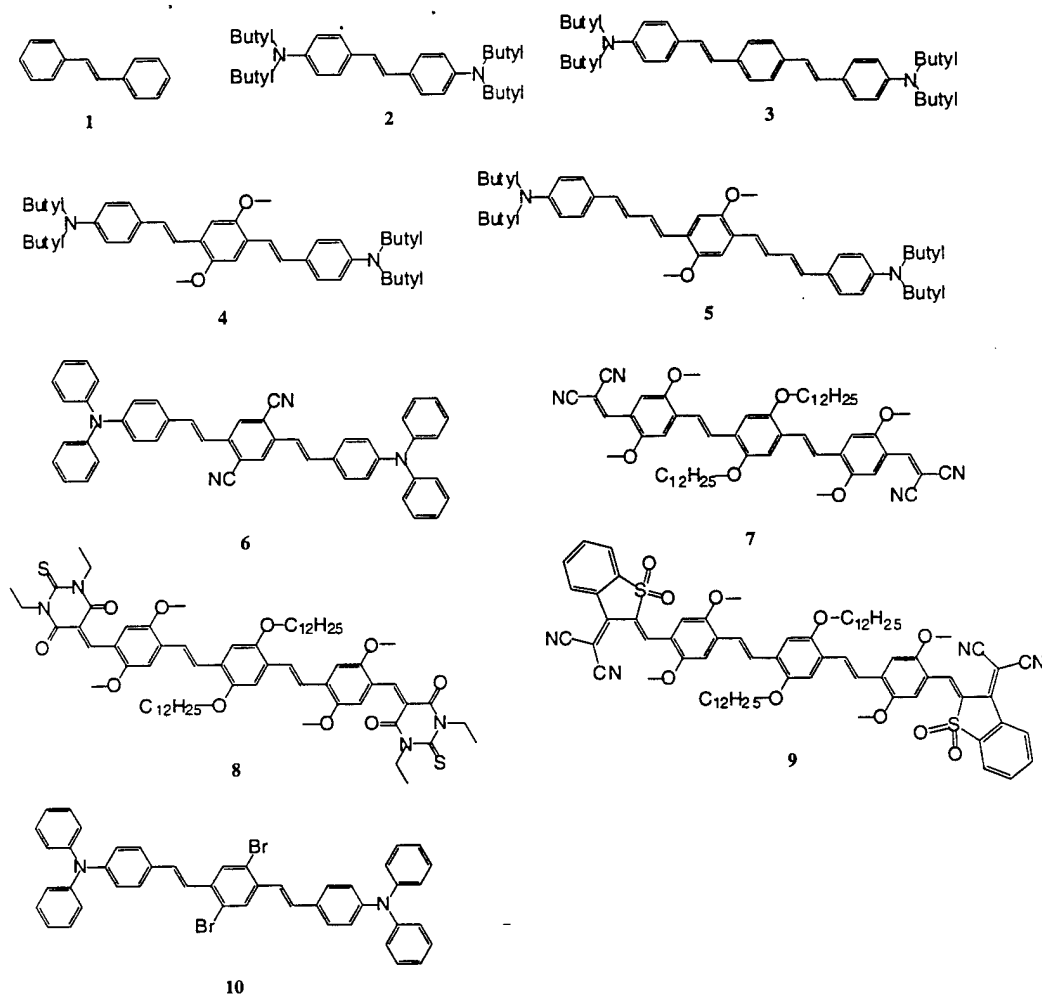
Figure 1: Structures and numbering scheme for compounds studied in this report.

As can be seen in Table 1, the calculations predict roughly an order of magnitude enhancement in δ upon substitution of *trans*-stilbene with terminal dimethylamino groups, consistent with the experimental data presented above. This two-photon transition is from the ground-state (S_0, $1A_g$) to the lowest excited state with A_g symmetry (S_2, $2A_g$). For both molecules, the S_2 state is located at about 0.8 eV above the lowest one-photon allowed excited state (S_1, $1B_u$), see Figure 2. A simplified form of the SOS expression for the peak two-photon resonance value of $\delta(\omega)$ for the $S_0 \rightarrow S_2$ transition, $\delta_{S_0 \rightarrow S_2}$, is

$$\delta_{S_0 \rightarrow S_2} \propto \frac{M_{01}^2 \, M_{12}^2}{(E_1 - E_0 - \hbar\omega)\Gamma^2} \qquad (2)$$

Table 1. Calculated and experimental two-photon optical data (wavelengths for the two-photon fluorescence excitation maxima (TPA λ_{max}) and two-photon excitation cross sections (δ)) for compounds **1-7**. The uncertainty in the experimental δ values is estimated to be ± 15%. Single photon absorption maxima (λ_{max}), wavelength for single photon emission maxima (Emission λ_{max}) and fluorescence quantum yields (Φ_f) are also given for compounds in this study. Two-photon fluorescence data were measured using nanosecond pulses and femtosecond pulses (in parentheses). For the theoretical results, **1'-7'** are model compounds for **1-7** wherein alkyl groups on amino or alkoxy groups and phenyl groups on terminal amino groups were replaced by methyl groups.

Theoretical results			Experimental data					
	TPA λ_{max}	δ		TPA λ_{max}	δ	λ_{max}	Emission λ_{max}	Φ_f
Comp #	nm	10^{-50} cm^{4}s/ photon	Comp #	nm	10^{-50} cm^{4}s/ photon	nm	nm	
1'	466	27.5	**1**	514 [ref. 13]	12 [ref. 13]			
2'	529	202.4	**2**	605 (<620)	210 (110 @ 620 nm)	374	410	0.90
3'	595	680.5	**3**	730 (~725)	995 (635)	408	455,490	0.88
4'	599	670.3	**4**	730 (~725)	900 (680)	428	560	0.88
-	620	712.5	**5**	775 (~750)	1250 (1270)	456	560	0.12
6'	625	950.0	**6**	835 (810)	1940 (3670)	472	525	0.86
7'	666	570.4	**7**	825 940 (815) (910)	480 620 (650) (470)	513	580	0.82
-	-	-	**8**	970	1390	554	641	0.06
-	-	-	**9**	975	4700	618	745	0.0055
-	-	-	**10**	~800	390	424	532	0.38·

where the subscripts 0, 1 and 2 refer to S_0, S_1, and S_2 states, $\hbar\omega = (E_2 - E_0)/2$. This expression results from taking S_1 as the dominant intermediate state and equating the damping factors for the $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions. Based on the results of the calculations, as illustrated in Figure 2, we can rationalize the increase in $\delta_{S_0 \rightarrow S_2}$ on going from *trans*-stilbene to 4,4'-bis(dimethyl)amino-*E*-stilbene on the basis of: (i) an increase in the S_1 to S_2 transition dipole moment (M_{12}) from 3.1 D to 7.2 D, (ii) an increase in the S_0 to S_1 transition dipole moment (M_{01}) from 7.1 D to 8.8 D, and (iii) a decrease in the one-photon detuning term, ($E_1 - E_0 - \hbar\omega$), from 1.8 eV to 1.5 eV. This enhancement results from the electron donating properties of the terminal amino groups. The calculations also show that the electronic excitation from S_0 to S_1 is accompanied by a substantial charge transfer (~0.14 e) from the amine groups to the central vinyl linkage, as we had hypothesized, leading to a large change in quadrupole moment upon excitation. This pronounced redistribution of the π-electronic density is correlated with an increase of electron delocalization in the first excited-state and results in a significant increase in the S_1 to S_2 transition dipole moment, which is the major contributor to the enhanced value of 4,4'-bis(dimethyl)amino-*E*-stilbene with respect to that of *trans*-stilbene. Another consequence of the terminal substitution with electron donors is a shift of the position of the two-photon resonance to lower energy.
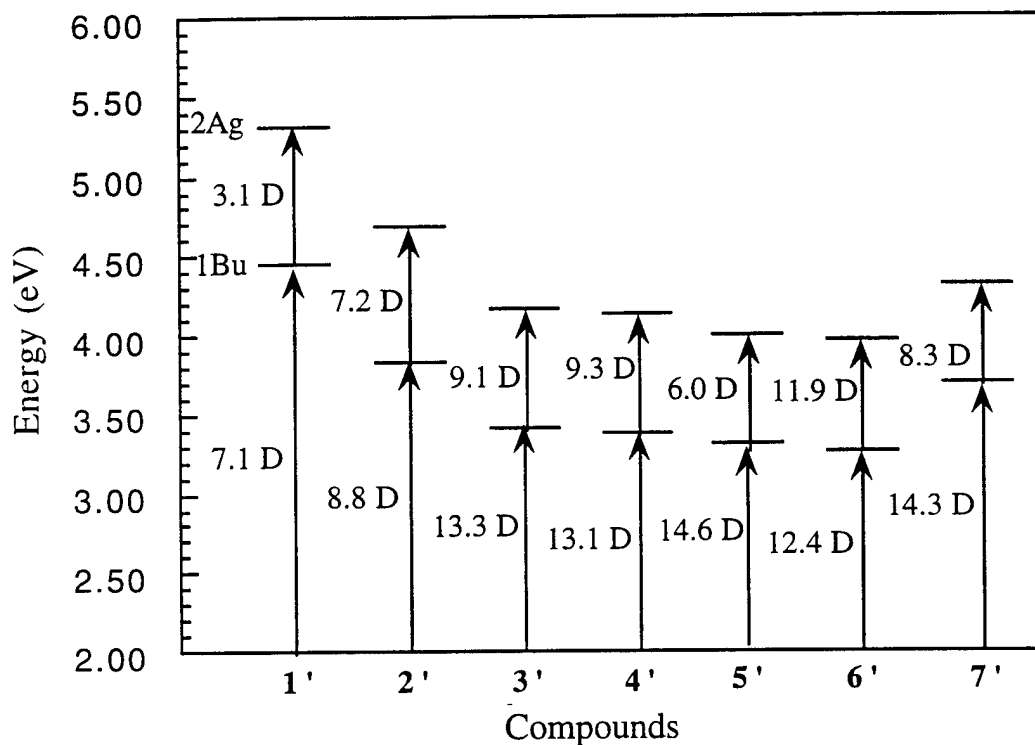
5

Figure 2: Scheme of the calculated energy levels and transition dipole moments for the three lowest singlet states for compounds closely related to **1'- 7'** (which are model compounds for **1- 7** wherein alkyl groups on amino or alkoxy groups and phenyl groups on terminal amino groups were replaced by methyl groups).

These results suggested several strategies to enhance δ and tune the wavelength of the two-photon absorption peak for π-conjugated organic molecules. Since the symmetric charge transfer and change in quadrupole moment appear to be important, we reasoned that structural features that could further enhance the change in quadrupole moment upon excitation could be beneficial for enhancing the corresponding transition dipole moments and the magnitude of δ. We therefore examined both theoretically and experimentally molecules in which: (i) the conjugation length was increased by inserting phenylene-vinylene or phenylene-butadienylene groups (compounds **3 - 5**) to increase the distance over which charge may be transfered; (ii) electron accepting cyano groups were attached to the central ring of the bis-styrylbenzene backbone (compound **6**), creating a "donor-acceptor-donor" motif, to increase the extent of charge transfer from the ends of the molecule to the center, and (iii) the sense of the symmetric charge transfer was reversed by

substituting electron donating alkoxy donors on all three rings of the bis-styrylbenzene and attaching relatively strong accepting dicyano vinyl (compound **7**), thiobarbituric acid (compound **8**), or 3-(dicyanomethylidenyl)-2,3-dihydrobenzothiophene-2-ylidenyl-1,1-dioxide (compound **9**) electron accepting groups on both ends, creating "acceptor-donor-acceptor" compounds. Finally, we were interested in introducing heavy atoms (for example bromine atoms, compound **10**) with a large spin–orbit coupling to facilitate intersystem crossing from the S_1 state to the lowest excited triplet state, T_1, aiming to create a two-photon absorbing molecule that could act as an efficient triplet sensitizer.

We performed the same INDO-MRD-CI calculations described above on a series of model compounds (**3'**, **4'**, **6'**, and **7'**) for **3**, **4**, **6**, and **7** wherein alkyl groups on amino or alkoxy groups and phenyl groups on terminal amino groups were replaced by methyl groups. The results which are shown in Table 1 support our proposed design strategies. They clearly show that increasing the conjugation length of the molecule or increasing the extent of symmetrical charge transfer from the ends of the molecule to the middle or *vice versa* leads to a large increase of δ and a shift of the two-photon absorption peak to longer wavelength relative to that of *trans*-stilbene.

We synthesized **3 - 10** by standard techniques and characterized them by nuclear magnetic resonance, electronic absorption, fluorescence and mass spectroscopies, as well as elemental analysis (the details of which will be described elsewhere). The two-photon absorption cross sections of these molecules were measured using the two-photon fluorescence excitation method with nanosecond (*19*) and femtosecond (*11*) laser pulses. In both cases, measurements were performed using fluorophores with well characterized δ-values as reference standards. (*11*) The positions and magnitudes of the two-photon resonances, the fluorescence quantum yields, and positions of the one-photon absorption bands are shown in Table 1.

Several important conclusions can be drawn from the data in Table 1: (i) there is reasonably good agreement between the peak values of δ measured using femtosecond and nanosecond pulses, and the INDO-MRD-CI calculations. (ii) The INDO-MRD-CI calculations reproduce the trends in the evolution of the two-photon absorption peak energies (although, as expected, the absolute excitation energies are systematically somewhat overestimated by theory, due to overcorrelation of the ground state using the MRD-CI scheme). (iii) Increasing the length of the molecule leads to a significant increase in δ, as can be seen by comparing results for **3**, **4**, and **5** with **2**. (iv) Our hypothesis that donor-acceptor-donor and acceptor-donor-acceptor compounds should have enhanced δ is borne out by the observation of large δ-values, in the range of 1400- 4500 x 10^{-50} cm^4sec/photon, for **6 - 9** relative to bis-1,4-(2-methylstyrl)benzene for which δ is 55 x 10^{-50} cm^4sec/photon. (*20*) (v) There are significant shifts of the peak position of the two-

photon absorption to longer wavelength upon increasing both the conjugation length and the extent of symmetric charge transfer. (vi) With the exceptions of **5, 8, 9**, and **10**, the compounds have very high fluorescence quantum yields indicating that they could be of interest as fluorescent probes for two-photon microscopy. (vii) The dibromo substituted compound **10** has a reasonably large δ-value, and its fluorescence quantum yield is low in comparison to **3**, consistent with efficient intersystem crossing. Preliminary results indicate that **10** is a singlet O_2 sensitizer, which makes it a good candidate for cytotoxicity and photodynamic therapy studies in biological tissues (*21*).

In summary, our experimental results and quantum chemical calculations provide strong support for the concept that molecules that undergo a symmetric charge redistribution from the ends of the molecule towards its center upon excitation, can exhibit large two-photon absorptivities. By exploiting this concept, we have designed and synthesized molecules with large δ values and high fluorescence quantum yields. These studies provide a clear set of design guidelines for the synthesis of new molecules with large two-photon absorptivities. In particular, we suggest that conjugated molecules with large changes of quadrupole moment upon excitation are worthy of examination in this regard. The large two-photon absorption cross sections of the molecules reported here, and molecules derived from the design strategies described, should greatly facilitate a variety of applications of two-photon excitation in biology, medicine, three- dimensional optical memory, photonics, (*22*) optical limiting (*5*) and materials science (*22*).

References and footnotes

1. W. Denk, J. H. Strickler, W. W. Webb, *Science*, **248**, 73 (1990).
2. R. M. William, D. W. Piston, W. W. Webb, *FASEB Journal*, **8**, 804 (1994).
3. W. Denk, K. Svoboda K, *Neuron*, **18**, 351 (1997).
4. R. H. Kohler, *et al.*, *Science.*, **276**, 2039 (1997).
5. J. E. Ehrlich, *et al.*, *Opt. Lett.*, **22**, 1843 (1997).
6. A. A. Said, *et al.*, *Chem. Phys. Lett.*, **228**, 646 (1994).
7. G. S. He, R. Gvishi, P. N. Prasad and B. Reinhardt, *Opt. Commun.*, **117**, 133 (1995).
8. D. A. Parthenopoulos, P. M. Rentzepis, *Science*, **245**, 843 (1989).
9. J. H. Strickler, W. W. Webb, *Opt. Lett.*, **16**, 1780 (1991).
10. W. Denk, *Proc. Nat. Acad. Sci.*, **91**, 6629 (1997).
11. C. Xu and W. W. Webb, *J. Opt. Soc. Am. B*, **13**, 481 (1996).
12. C. Xu, *et al.*, *Proc. Nat. Acad. Sci.*, **93**, 10763 (1996).

13. R. J. M. Anderson, G. R. Holtom, W. M. McClain, *J. Chem. Phys.*, **70**, 4310-4315 (1979).

14. M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. J. P. Stewart, *J. Am. Chem. Soc.*, **107**, 3902 (1985).

15. J. Ridley, M. Zerner, *Theoret. Chim. Acta*, **32**, 111 (1973).

16. R. J. Buenker, S. D. Peyerimhoff, *Theoret. Chim. Acta*, **35**, 33 (1974).

17. B. Dick, R. M. Hochstrasser, H. P. Trommsdorff, "Nonlinear Optical Properties of Organic Molecules and Crystals," Vol. 2, D. S. Chemla and J. Zyss, Eds. (Academic Press, Orlando, 1987) pp. 167-170.

18. B. J. Orr, J. F. Ward, *Mol. Phys.*, **20**, 513 (1971).

19. The two-photon absorption cross-sections were determined via two-photon excited fluorescence measurements, using 5ns, 10Hz pulses from a Nd:YAG-pumped optical parametric oscillator/amplifier system. The set-up consisted of two arms to allow relative measurements with respect to bis-1,4-(2-methylstyrl)benzene (in cyclohexane), fluorescein (in pH 11 water) and rhodamine B (in methanol). The sample chromophores were dissolved in toluene (10^{-4}M concentration); an approximately collimated beam was used to excite the samples over the 1cm length of the cuvettes. The fluorescence emitted at right angles to the excitation was collected and focused onto photomultiplier tube detectors. The intensity of the incident beam was adjusted to assure excitation in the intensity-squared regime.

20. S. M. Kennedy, F. E. Lytle, *Anal. Chem.*, **58**, 2643 (1986); We have scaled the peak δ value reported in this paper by a factor chosen to match the long wavelength data reported therein with that of ref. 11.

21. M. Lipson, M. Levin, S. R. Marder, J. W. Perry, unpublished results.

22. B. M. Cumpston *et al.*, submitted.

Equipment purchased under DURIP grant

Vendor

Instruments S.A. Inc. JOBIN YVON SPEX

Spex Model FL112 High Performance Research Grade Fluorimeter, with variable entrance and exit slit, 450W Lamp and Power supply, R2658 Detector, Datamax software Gram 386 processor.

Total   66,876.87 (including 8.25% CA sales tax)

Vendor:

Spectra Physics Lasers, Inc.

Nd:YAG Laser, 1500 mJ at 1064 nm, 10 Hz	PRO-250-10	69,950
Active stabilization for PRO-250-10	BeamLok	9,800
Narrowband parametric oscillator	MOPO-730	64,950
Enhanced energy option for PRO-250-10	EEO-4-355	30,950
Computer Interface for MOPO-730	IEEE-488	1,250
Mounted 532 nm dichroic mirrors	IHS-532	2,500
Beam Dump BD-5		600

Total	180,000
less Patlex	-4,545
less discount	-25,455
Total	150,000
Total w/CA	162,380